Fractalkine (CX3CL1) as a Diagnostic Marker for Childhood Onset Systemic Lupus Erythematosus

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Abstract
Background: - more specific, sensitive, and in the same time non-invasive indicators for the early prediction of juvenile SLE are required.
Aim: - to evaluate serum fractalkine (CX3CL1) as a diagnostic marker, predictor of nephritis, and indicator of disease activity in pediatric SLE.
Methods: - study included 41 children newly diagnosed with SLE, age ranged from 5 to 18 years, 24 (58.5%) were presented lupus nephritis "diagnosed by renal biopsy". A total of 20 healthy age and sex matched children were included as controls. Serum Fractalkine levels were measured using human CX3CL1 ELISA.
Results: - Serum Fractalkine were significantly higher in SLE patients (median = 1323, range 591- 16547 IU/ml) than healthy controls (median = 950, range 591- 1583 u/ml) p=0.001. Serum Fkn can be a significant diagnostic marker for juvenile lupus indicated by the are under the ROC curve, AUC = 0.81 (95% confidence interval [CI], 0.70-0.92, P<0.001), at the level 1213 IU/ml serum Fkn detected SLE with sensitivity 0.78 (95% CI, 0.62 - 0.89), specificity 0.91 (95% CI, 0.71 – 0.99), positive predictive value 0.94 (95% CI, 0.80 – 0.98), negative predictive value 0.67 (95% CI, 0.55- 0.80), and accuracy 0.82 (95% CI, 0.71 – 0.91). Serum levels of Fkn showed no statistically significant differences when compared in patients with lupus nephritis against patients without nephritis, and was not correlated with disease activity.
Conclusions: - Serum level of Fractalkine can be a diagnostic marker for childhood onset SLE either with or without lupus nephritis, with no significant correlation with activity status or the stage of LN.
Keywords: Biomarkers, CX3CL1, Fractalkine, pediatric lupus nephritis, systemic lupus erythematosus.

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with multi-organ affection. Pediatric, juvenile, or childhood onset SLE (cSLE) is a rare disease, with an incidence of 0.3-0.9/100,000 children per year and prevalence of 3.3-8.8/100,000 children [1]. Although similar to the manifestations observed in adults, the clinical events present in cSLE are usually more severe and involve multiple organs [2]. Renal involvement occurs in 50-75% of pediatric patients with SLE [3].

Serum and urine markers widely used for the diagnosis of juvenile SLE such as the anti-double stranded DNA, complement, and erythrocyte sedimentation rate often lack specificity and sensitivity. Therefore, noninvasive, more specific and sensitive indicators for the early prediction of SLE are required[4].

Chemokines have important roles in the recruitment and retention of inflammatory immune cells to the sites of inflammation [5].Understanding the role of chemokines in the pathogenesis of autoimmune diseases guides the new targeted therapy approaches. The chemokine CX3CL1 is the only member of CX3C chemokine subclass, the soluble form of Fractalkine (Fkn; CX3CL1) exerts a chemotactic effect on inflammatory/immune cells [4].

CX3CL1 and its receptors CX3CR1 are implicated in the development of several autoimmune rheumatic diseases like; rheumatoid arthritis [6-9], Sjögren's syndrome [10, 11], Scleroderma [12, 13], and systemic lupus erythematosus[14, 15]. In addition, Fkn and its receptors have been demonstrated to be potential targets of therapy in inflammatory disorders, indicated by the ability of anti-Fkn monoclonal antibodies to reduce arthritis and synovial destruction[6, 16], and the diminished lupus nephritis symptoms in mice after the administration of Fkn antagonists [17]. Furthermore, TNF antagonists have shown to suppress the TNF-α induced CX3CL1 expression in human umbilical vein endothelial cells through the regulation of NF-κB pathway[18, 19].

Although Fkn has been studied previously in SLE patients, it has not been studied in juvenile SLE; this encouraged us to carry out this research for better understanding of its role in pathogenesis, and to evaluate its sensitivity and accuracy to detect SLE in children.

Subjects and methods

This prospective case control study was conducted in the Clinical pathology and Pediatrics departments, faculty of medicine, Zagazig University, Al-sharquia governorate, Egypt, from January 2019 to January 2020. Consecutive children >1 and ≤ 18 years from both genders and the same Egyptian ethnicity that were newly diagnosed as juvenile SLE "diagnosed according to the new 2019 European league against rheumatism/American college of rheumatology classification criteria for systemic lupus erythematosus (EULAR)" were involved. Patients whose parents disagree to provide written informed consent, who suffer from other rheumatologic or non-rheumatologic diseases or who started therapy were excluded.

Subjects

This study included 41 children newly diagnosed with SLE, their age ranged from 5 to 18 years, 24 (58.5%) were presented with lupus nephritis "diagnosed by renal biopsy based on the international

Society of Nephrology and the Renal Pathology Society classifications [20],. A total of 20 healthy age and sex matched children were included as controls.

Ethical consideration

Zagazig University-Institutional Research Board (ZU-IRB) approved this work; approval number (4852/10-9-2018). Written informed consent was obtained from the parents of the children involved in the study. All procedures were carried out according to the Declarations of Helsinki.

Sample size calculation

Due to rarity of the disease, all cases that fulfill the inclusion criteria admitted to hospital within the duration of the study were included.

Methods

Measurement of Fractalkine serum levels

Blood samples were collected from patients after diagnosis; sera were separated and stored at −20°C till analysis, serum Fractalkine levels were assessed using human CX3CL1 ELISA kit (Nova Lifetech Inc. Hong Kong, China) that applies sandwich ELISA technique.

Assessment of SLE activity status

The Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) was used to assess the activity status in patients using the data obtained from history taking and clinical examinations and laboratory investigation results performed upon diagnosis [21].

Statistical analysis

All data were entered and analyzed with SPSS version 22.0 (IBM Corp., Chicago, IL, USA). Shapiro–Wilk test was performed. Normally distributed data are presented as means ± standard deviation and non-normally distributed data are presented as medians and ranges. The t-test was used to compare means; Mann-whitny and Kruskal-wallis tests were used to compare medians. Categorical variables were compared using the Chi square test and Fisher's exact test when frequencies are less than five. A receiver operating characteristic (ROC) curve was constructed to calculate the area under curve (AUC) at 95% CI and to obtain the best cutoff value. Correlations between variables were tested using the Spearman correlation coefficient (r). Results were considered significant when $p < 0.05$.

Results

A total of 41 newly diagnosed juvenile SLE patients were enrolled in this study, with median age (range) 13.0 (5-18) years and female predominance 37 (90.2%). 24 (58.5%) of patients were complicated with lupus nephritis confirmed by renal biopsy. Demographic features and symptoms of patients are summarized in **Table 1**. Arthralgia was the most frequent symptom in both systemic lupus with and without nephritis patients, fever and edema were the most frequent symptoms in lupus without nephritis and lupus nephritis patients, respectively.

Serum levels of Fractalkine were significantly higher in SLE patients (median = 1323, range 591-16547 IU/ml) than healthy controls (median = 950, range 591- 1583 u/ml) p=0.001.

Serum Fkn is a significant diagnostic marker for juvenile lupus indicated by the are under the ROC curve, AUC = 0.81 (95% confidence interval [CI], 0.70-0.92, P<0.001), the level with the best accuracy obtained from the output data was 1213 IU/ml, at this cutoff serum Fkn detected SLE with sensitivity 0.78 (95% CI, 0.62 - 0.89), specificity 0.91 (95% CI, 0.71 – 0.99), positive predictive value 0.94 (95% CI, 0.80 – 0.98), negative predictive value 0. 67 (95% CI, 0.55- 0.80), and accuracy 0.82 (95% CI, 0.71 – 0.91).

Serum levels of Fkn in patients with lupus nephritis (median = 1389, range 711- 16547 IU/ml) and in SLE patients without nephritis (median = 1429, range 591- 4700 IU/ml) showed no statistically significant differences, P = 0.4. In addition, serum Fkn levels in lupus nephritis patients had no significant correlation with either 24 hours protein in urine, CRP, ESR, serum creatinine, C3 and C4 complement levels, or the stage of lupus nephritis, data are presented in **Table 2** and **Table 3.**

The median (range) disease activity (SLEDAI-2K) in this cohort were 12 (4-25). Activity in lupus nephritis patients were significantly higher than in patients without nephritis, median (ranges) were 14 (7-25) versus 8 (4-17), respectively, p=0.001. Serum levels of Fkn showed no significant correlation with the activity of the disease in all patients, r= - 0.008; p=0.9, and in those without and with LN separately, r= 0.1; p=0.6 and r= -0.3; p= 0.1, respectively.

Table 1; Demographic features and symptoms of patients

	Lupus without nephritis n=17	Lupus with nephritis n=24	P
Age (years)			
Mean ± SD	13.5 ± 3.4	12.4 ± 2.4	0.4
Range	5 - 18	9 – 19	
Gender; n (%)			
Male	2 (11.8%)	2 (8.3%)	0.6
Female	15 (88.2%)	22 (91.7%)	
Symptoms [a]; n (%)			
Arthralgia	7(41.1%)	9(37.5%)	
Bleeding	3(17.6%)	2(8.3%)	
In ability to walk	5(29.4%)	3(12.5%)	
Fever	7(41.1%)	1(4.2%)	
Skin Rash	4(23.5%)	3(12.5%)	
Cold like symptoms	2(11.8%)	0(0.0%)	
Vomiting	3(17.6%)	2(8.3%)	N.S [b]
Oral ulcer	2(11.8%)	0(0.0%)	
Puffiness	2(11.8%)	0(0.0%)	
Edema	2(11.8%)	6(25.0%)	
Abdominal pain	1(5.9%)	2(8.3%)	
Pallor	1(5.9%)	0(0.0%)	
Squint	1(5.9%)	0(0.0%)	
Purpura	1(5.9%)	2(8.3%)	

Loss of appetite	1(5.9%)	0(0.0%)
Jaundice	0(0.0%)	2(8.3%)
Fatigue	0(0.0%)	1(4.2%)

Student t-test, Chi square and fisher's exact t test were performed

[a] Patients are presented with multiple symptoms

[b] P value for each symptom showed no significant differences

Table 2; Correlations of Fractalkine with other numeric variables

Variable	r	P
Serum creatinine	0.1	0.4
24h urine protein	-0.1	0.9
CRP	-0.004	0.9
C3	0.09	0.5
C4	0.01	0.9
ESR	0.2	0.9
SLEDAI-2X	0.01	0.9

Spearman correlation coefficient test was performed

CRP: C reactive protein, C3: complement 3, C4: complement 4, ESR: erythrocyte sedimentation rate, h: hours. SLEDAI-2X: Systemic Lupus Erythematosus Disease Activity Index 2000

Table 3; Serum Fractalkine in stages of lupus nephritis

lupus nephritis (n=24)	Number (%)	Serum fractalkine (IUml) Median (range)	P value
Class II	6 (25.0)	2067 (711-16547)	
Class III	7 (29.2)	1229 (711-3822)	
Class II with early class III changes	3 (12.5)	2865 (1309-11082)	0.2
Class IV	7 (29.2)	1229 (830-2306)	
Class V	1 (4.2)	5697	

Discussion

Systemic lupus erythematosus is a complex and highly diverse autoimmune disease either clinically or serologically, rendering the search for new biomarkers for diagnosis, follow-up, and poor risk prediction is mandatory as an effort to minimize the chance of over or under-diagnosis of the disease [22]. Although the new classification criteria for SLE (EULAR/ACR) has reached 0.98 sensitivity and 0.96 specificity at 95% confidence interval, the inclusion of novel biomarkers beside autoantibodies is still recommended to improve the performance of SLE classification. In addition, the evaluation of biomarkers against these criteria is considered as an important area for future research[23].

The gender distribution of our patients showed a female predominance with female to male (F:M) ratio 9.25:1, when patients were further subdivided according to age; the ratio became 7:2 and 30:2 (15:1) in children in the first and second decades of life, respectively.

These ratios were not concordant with the overall distribution of childhood onset SLE (4:3 and 4:1) in the 1st and 2nd decades, respectively [24], but goes with the results of a previous study performed on another Egyptian cohort, the ratio in juvenile patients was 39:1, authors attributed this to the inclusion of adolescent females in their work, in addition, other regional, ethnic and socio-economic factors that lead to widespread vitamin D deficiency in growing women may be responsible for the skewed results toward female predominance [25].

Serum Fractalkine (CX3CL1) as a biomarker for cSLE diagnosis

In this study serum Fkn levels were significantly higher in all children with SLE when compared with healthy children; serum Fkn > 1213 IU/ml had a high specificity and a moderate sensitivity to SLE, to the best of our knowledge; this is the first prospective study to evaluate serum Fkn in juvenile SLE worldwide at least till the time of writing this manuscript, and it has been evaluated in adults in few literatures. Yajima et al [26] and Guo et al [27] reported that serum Fkn was significantly higher in SLE patients than in the healthy controls.

The EULAR/ACR classification criteria was re-evaluated in cSLE by Rodrigues et al[28], although it showed a better diagnostic performance for SLE in adults, authors found that its sensitivity and specificity decreased in children to be 87.7% and 67.4%, respectively, then they suggested to increase the cutoff score to be ≥13 instead of 10, this modification could increase the specificity and the accuracy of this system in children with SLE.

Moreover, other limitations of the EULAR/ACR include; the probability of misclassifying patients with overlapping syndromes, the non-inclusion of novel biomarkers, and the exclusion of ANA negative patients from the entry into classification.

The detection of anti-dsDNA antibodies is the most useful laboratory test used to confirm SLE diagnosis due to its high SLE specificity. Although, it's mean sensitivity is only 57.3%. In addition, the tendency of anti-dsDNA antibody titers to fluctuate during the disease course is another limitation of this test [29]. The aforementioned data highlights the value of searching for and evaluating novel biomarkers for the diagnosis of SLE. Serum Fkn can be considered as a possible biomarker for SLE classification that warrants further evaluation and studying.

Serum Fractalkine (CX3CL1) in SLE children with lupus nephritis

In this cohort serum Fkn did not predict the occurrence of lupus nephritis; moreover, there were no significant correlation with the histopathology of renal affectation. These results are not concordant with previous studies; in an experimental study performed on lupus mice model; Nakatani et al detected the increased CX3CL1 mRNA and protein expression in glomeruli of (SCID) mice with proliferative lupus nephritis [30]. In another study; fractalkine/CX3CL1 antagonist could significantly reduce glomerular hypercellularity, sclerosis, crescent formation and vacuities when administered to (MRL/lpr) mice with early lupus nephritis [31]. In the same context; Qing et al [32] stated that pathogenic anti ds.DNA antibodies induce increased expression of CX3CL1 gene in (MRL/lpr) mice model, with

further increase with the progression of nephritis, Menke et al[33] detected the increased expression of CX3CL1 and its receptors CX3CR1 in glomerular tissue of mice from the same model. On the other hand; Schiffer et al [34] described that CX3CL1 gene was not upregulated in relation to disease progression in NZB/NZW female mice model, this conflict was attributed to the difference in the lupus prone mouse models [35].

In human subjects; Yoshimoto et al [36] analyzed frozen sections from 49 patents with LN, they detected increased glomerular expression of Fkn and accumulation of CD16 monocytes/macrophages, which are correlated with disease activity and proliferative lesions.

One study considered the soluble form of Fkn; where the urine and serum levels of fractalkine were significantly higher in patients with proliferative LN (Class III and class IV) and lower in patients with non-proliferative LN (class V)[37].

Correlation analysis of Serum Fractalkine (CX3CL1) and SLEDAI

Serum Fkn was not correlated with disease activity in this study in either group of patients. In contrast, Lan et al [37] reported highly significant correlation of serum and urine Fkn with activity status in LN patients.

The conflict between previous literatures and our results can be attributed to different study populations regarding the age of onset of SLE, Mina and Brunner [38]outlined the differences in the pathogenesis and course of disease in between adult with SLE and c SLE, and postulated that the underlying genetic variations and its consequent biological effects can explain the noted phenotypic differences between cSLE and adults SLE.

This is a preliminary study that draws attention to the evaluation of serum Fkn as a diagnostic marker of cSLE which requires thorough examination with larger sample size, also; urinary Fkn is a candidate marker the may harbor diagnostic and prognostic value in these patients.

Conclusions

Serum level of Fractalkine can be a diagnostic marker for childhood onset SLE either with or without lupus nephritis, with no significant correlation with activity status or the class of LN.

Limitations and recommendations

The sample size is relatively small and only healthy controls are included, further studies with larger sample size and with the inclusion of patients with other autoimmune and inflammatory disorders as disease controls are recommended.

Declarations

Financial Disclosure: All the authors have indicated they have no financial relationships relevant to this article to disclose.

Competing interest. All the authors have no competing interest to declare.

Ethical consideration

Ethical approval for this study was obtained from Zagazig University-Institutional Research Board (ZU-IRB), approval number (4852/10-9-2018). All procedures were performed according to the Declaration of Helsinki.

Consent to participate: a signed informed consent was taken from parents of the involved children.

Consent for publication: Not applicable.

Code availability: Not applicable.

Data availability: data are available on reasonable request.

Author Contributions; The requirements for authorship have been met. All authors contributed to the study conception and design. The original idea was the first author's; the last author contributed to patient's selection, history taking, data collection, blood sample withdrawal, sample processing, results collection, and data analysis; under the supervision of the first three authors. The first author contributed to manuscript writing and submission for publication. Authors certify that we have personally written at least 90 percent of the manuscript. Finally, the manuscript has been read and approved by all the authors. All authors are responsible for reported research.

References

1.   Kamphuis, S. and E.D. Silverman, Prevalence and burden of pediatric-onset systemic lupus erythematosus. Nature Reviews Rheumatology, 2010. **6**(9): p. 538.

2.   Bennett, M. and H.I. Brunner, Biomarkers and updates on pediatrics lupus nephritis. Rheumatic diseases clinics of North America, 2013. **39**(4): p. 833.

3.   Levy, D.M. and S. Kamphuis, Systemic lupus erythematosus in children and adolescents. Pediatric Clinics, 2012. **59**(2): p. 345-364.

4.   Lan, L., F. Han, X. Lang, and J. Chen, Monocyte chemotactic protein-1, fractalkine, and receptor for advanced glycation end products in different pathological types of lupus nephritis and their value in different treatment prognoses. PLoS One, 2016. **11**(7): p. e0159964.

5.   Charo, I.F. and R.M. Ransohoff, The many roles of chemokines and chemokine receptors in inflammation. N Engl J Med, 2006. **354**(6): p. 610-21.

6.   Nanki, T., Y. Urasaki, T. Imai, M. Nishimura, K. Muramoto, T. Kubota, et al., Inhibition of fractalkine ameliorates murine collagen-induced arthritis. J Immunol, 2004. **173**(11): p. 7010-6.

7.   Ruth, J.H., M.V. Volin, G.K. Haines, 3rd, D.C. Woodruff, K.J. Katschke, Jr., J.M. Woods, et al., Fractalkine, a novel chemokine in rheumatoid arthritis and in rat adjuvant-induced arthritis. Arthritis Rheum, 2001. **44**(7): p. 1568-81.

8.   Koch, A.E., Chemokines and their receptors in rheumatoid arthritis: future targets? Arthritis Rheum, 2005. **52**(3): p. 710-21.

9.   Klosowska, K., M.V. Volin, N. Huynh, K.K. Chong, M.M. Halloran, and J.M. Woods, Fractalkine functions as a chemoattractant for osteoarthritis synovial fibroblasts and stimulates phosphorylation of mitogen-activated protein kinases and Akt. Clin Exp Immunol, 2009. **156**(2): p. 312-9.

10. Tsubota, K., T. Nishiyama, K. Mishima, H. Inoue, T. Doi, Y. Hattori, et al., The role of fractalkine as an accelerating factor on the autoimmune exocrinopathy in mice. Invest Ophthalmol Vis Sci, 2009. **50**(10): p. 4753-60.

11. Wildenberg, M.E., C.G. van Helden-Meeuwsen, H.A. Drexhage, and M.A. Versnel, Altered fractalkine cleavage potentially promotes local inflammation in NOD salivary gland. Arthritis Res Ther, 2008. **10**(3): p. R69.

12. Gabrielli, A., E.V. Avvedimento, and T. Krieg, Scleroderma. N Engl J Med, 2009. **360**(19): p. 1989-2003.

13. Hasegawa, M., S. Sato, T. Echigo, Y. Hamaguchi, M. Yasui, and K. Takehara, Up regulated expression of fractalkine/CX3CL1 and CX3CR1 in patients with systemic sclerosis. Ann Rheum Dis, 2005. **64**(1): p. 21-8.

14. Yajima, N., T. Kasama, T. Isozaki, T. Odai, M. Matsunawa, M. Negishi, et al., Elevated levels of soluble fractalkine in active systemic lupus erythematosus: potential involvement in neuropsychiatric manifestations. Arthritis Rheum, 2005. **52**(6): p. 1670-5.

15. Nakatani, K., S. Yoshimoto, M. Iwano, O. Asai, K. Samejima, H. Sakan, et al., Fractalkine expression and CD16+ monocyte accumulation in glomerular lesions: association with their severity and diversity in lupus models. Am J Physiol Renal Physiol, 2010. **299**(1): p. F207-16.

16. Nanki, T., T. Imai, K. Nagasaka, Y. Urasaki, Y. Nonomura, K. Taniguchi, et al., Migration of CX3CR1-positive T cells producing type 1 cytokines and cytotoxic molecules into the synovium of patients with rheumatoid arthritis. Arthritis Rheum, 2002. **46**(11): p. 2878-83.

17. Nakayama, T., Y. Watanabe, N. Oiso, T. Higuchi, A. Shigeta, N. Mizuguchi, et al., Eotaxin-3/CC chemokine ligand 26 is a functional ligand for CX3CR1. J Immunol, 2010. **185**(11): p. 6472-9.

18. Scott, D.L. and G.H. Kingsley, Tumor necrosis factor inhibitors for rheumatoid arthritis. N Engl J Med, 2006. **355**(7): p. 704-12.

19. Garcia, G.E., Y. Xia, S. Chen, Y. Wang, R.D. Ye, J.K. Harrison, et al., NF-kappaB-dependent fractalkine induction in rat aortic endothelial cells stimulated by IL-1beta, TNF-alpha, and LPS. J Leukoc Biol, 2000. **67**(4): p. 577-84.

20. Tao, J., H. Wang, X.-J. Yu, Y. Tan, F. Yu, S.-X. Wang, et al., A Validation of the 2018 Revision of International Society of Nephrology/Renal Pathology Society Classification for Lupus Nephritis: A Cohort Study from China. American Journal of Nephrology: p. 1-10.

21. Gladman, D.D., D. Ibañez, and M.B. Urowitz, Systemic lupus erythematosus disease activity index 2000. The Journal of Rheumatology, 2002. **29**(2): p. 288-291.

22. Liu, C.C., A.H. Kao, S. Manzi, and J.M. Ahearn, Biomarkers in systemic lupus erythematosus: challenges and prospects for the future. Ther Adv Musculoskelet Dis, 2013. **5**(4): p. 210-33.

23. Aringer, M., K. Costenbader, D. Daikh, R. Brinks, M. Mosca, R. Ramsey-Goldman, et al., 2019 European League Against Rheumatism/American College of Rheumatology Classification Criteria for Systemic Lupus Erythematosus. Arthritis Rheumatol, 2019. **71**(9): p. 1400-1412.

24. Mina, R. and H.I. Brunner, Pediatric lupus--are there differences in presentation, genetics, response to therapy, and damage accrual compared with adult lupus? Rheumatic diseases clinics of North America, 2010. **36**(1): p. 53-viii.

25. Mohamed, D.F., A.B.E.-D.A. Aziz, S.A.-M. Hassan, N.H. Shedid, R.H. El-Owaidy, and M.A.E.M. Teama, Juvenile lupus: Different clinical and serological presentations compared to adult lupus in Egypt. The Egyptian Rheumatologist, 2018. **40**(1): p. 55-58.

26. Yajima, N., T. Kasama, T. Isozaki, T. Odai, M. Matsunawa, M. Negishi, et al., Elevated levels of soluble fractalkine in active systemic lupus erythematosus: Potential involvement in neuropsychiatric manifestations. Arthritis & Rheumatism, 2005. **52**(6): p. 1670-1675.

27. Guo, L., X. Lu, Y. Wang, C. Bao, and S. Chen, Elevated levels of soluble fractalkine and increased expression of CX3CR1 in neuropsychiatric systemic lupus erythematosus. Experimental and Therapeutic Medicine, 2017. **14**(4): p. 3153-3158.

28. Rodrigues Fonseca, A., M.C. Felix Rodrigues, F.R. Sztajnbok, M. Gerardin Poirot Land, and S. Knupp Feitosa de Oliveira, Comparison among ACR1997, SLICC and the new EULAR/ACR classification criteria in childhood-onset systemic lupus erythematosus. Advances in Rheumatology, 2019. **59**(1): p. 20.

29. Manzi, S., J.S. Navratil, M.J. Ruffing, C.C. Liu, N. Danchenko, S.E. Nilson, et al., Measurement of erythrocyte C4d and complement receptor 1 in systemic lupus erythematosus. Arthritis & Rheumatism, 2004. **50**(11): p. 3596-3604.

30. Nakatani, K., S. Yoshimoto, M. Iwano, O. Asai, K.-i. Samejima, H. Sakan, et al., Fractalkine expression and CD16+ monocyte accumulation in glomerular lesions: association with their severity and diversity in lupus models. American Journal of Physiology-Renal Physiology, 2010. **299**(1): p. F207-F216.

31. Inoue, A., H. Hasegawa, M. Kohno, M.R. Ito, M. Terada, T. Imai, et al., Antagonist of fractalkine (CX3CL1) delays the initiation and ameliorates the progression of lupus nephritis in MRL/lpr mice. Arthritis & Rheumatism, 2005. **52**(5): p. 1522-1533.

32. Qing, X., J. Zavadil, M.B. Crosby, M.P. Hogarth, B.H. Hahn, C. Mohan, et al., Nephritogenic Anti-DNA antibodies regulate gene expression in MRL/lpr mouse glomerular mesangial cells. Arthritis & Rheumatism, 2006. **54**(7): p. 2198-2210.

33. Menke, J., G.C. Zeller, E. Kikawada, T.K. Means, X.R. Huang, H.Y. Lan, et al., CXCL9, but not CXCL10, Promotes CXCR3-Dependent Immune-Mediated Kidney Disease. Journal of the American Society of Nephrology, 2008. **19**(6): p. 1177-1189.

34. Schiffer, L., R. Bethunaickan, M. Ramanujam, W. Huang, M. Schiffer, H. Tao, et al., Activated Renal Macrophages Are Markers of Disease Onset and Disease Remission in Lupus Nephritis. The Journal of Immunology, 2008. **180**(3): p. 1938-1947.

35. Liao, X., T. Pirapakaran, and X.M. Luo, Chemokines and Chemokine Receptors in the Development of Lupus Nephritis. Mediators of Inflammation, 2016. **2016**: p. 6012715.

36. Yoshimoto, S., K. Nakatani, M. Iwano, O. Asai, K. Samejima, H. Sakan, et al., Elevated levels of fractalkine expression and accumulation of CD16+ monocytes in glomeruli of active lupus nephritis. Am J Kidney Dis, 2007. **50**(1): p. 47-58.

37. Lan, L., F. Han, X. Lang, and J. Chen, Monocyte Chemotactic Protein-1, Fractalkine, and Receptor for Advanced Glycation End Products in Different Pathological Types of Lupus Nephritis and Their Value in Different Treatment Prognoses. PloS one, 2016. **11**(7): p. e0159964-e0159964.

38.   Mina, R. and H.I. Brunner, Update on differences between childhood-onset and adult-onset systemic lupus erythematosus. Arthritis research & therapy, 2013. **15**(4): p. 218-218.